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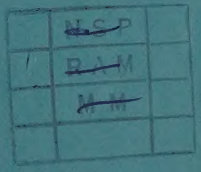

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Fifth Idian Microbiological Congress

Madras—1962.

The fifth Annual Congress and Scientific Session of the Association of Microbiologists of India will be held in the Madras Medical College from 2nd to 4th March, 1962. Apart from business meetings, there will be morning and afternoon sessions for *presentation of original papers and demonstrations*. Those willing to read papers are requested to send the titles and a 250 word summary of their proposed communications to the General Secretary not later than 1st February and the full paper by 15th February, 1962. In addition to reading of papers, *a Group Discussion on the Need for Centres of Type Culture Collection of Micro-organisms in India* and *a symposium on Microbial Variations* will be arranged. Contributors and participants wishing to show slides other than of the standard size ($3\frac{1}{2}'' \times 3\frac{1}{2}''$) should inform the General Secretary of the relevant details when submitting abstracts. A detailed programme of the meeting will be circulated to all members after it is finalized.

It has been decided to organize an *Exhibition of Scientific Instruments and Drugs* by interested firms during the Congress.

An Excursion trip to important places in and around Madras is likely to be arranged for the participants.

S. MUKERJEE
General Secretary

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OCCURRENCE OF *SALMONELLA* SP. IN POULTRY AND DOMESTIC ANIMALS

V. K. SHARMA AND C. M. SINGH

From the Department of Pathology and Bacteriology, Veterinary College, Mathura

(Received for publication, March 1961)

Pathak and Singh (1959) isolated *Salmonella enteritidis* and *Salmonella dublin* from poultry. This finding appeared to be of interest because of the possibility of the occurrence of unusual serotypes in birds and of its importance in public health. The present study deals with the occurrence of *Salmonella* sp. in domestic animals and birds.

Materials and Methods

Materials for the isolation of *Salmonella* sp. were collected from living and dead animals from the government poultry farm, Mathura; mechanised state farm, Madhurikund, Mathura; poultry farm Ingraham Institute, Ghaziabad; state livestock farm, Chak-ganjaria, Lucknow; central sheep and wool research station, Pashulok, Dehradun; Aurangabad and Damodarpura villages; sadar bazar locality; and slaughter house of Mathura.

Salmonella sp. were isolated by the methods of Edwards, Bruner and Moran (1948) and Smith and Buxton (1951). For enrichment media, Muller's tetrathionate broth, Kauffmann's modified tetrathionate broth with brilliant green and bile, selenite broth and hydroquinone broth were used. *Salmonella-Shigella* agar, brilliant green agar and desoxycholate citrate agar were used as selective media. In the beginning of the work the selective media contained lactose only but later on sucrose and salicin were also incorporated in the media. The media were prepared according to Mackie and McCartney (1956). The bacteria developing in selective media that appeared suspicious were examined for biochemical reactions, polyvalent O serum (A to E) test and *Salmonella*-genus-specific-bacteriophage lysis tests (Cherry, Davis, Edwards and Hogan, 1954) for their identity.

Serological typing was done by the senior author following the techniques of Edwards and Bruner (1942) and Kauffmann (1951).

Results

Out of 2,970 animals examined only 57 were found to harbour *Salmonella* sp.

No. of animals examined		No. of animals positive for *Salmonella* sp.
290	Buffaloes	9
455	Cattle	8
206	Dead embryos	7
110	Ducks	1
1,322	Fowls	24
91	Goats	4
68	Pigeons	Nil
247	Pigs	3
18	Rats	1
163	Sheep	Nil
Total 2,970		57

Seventeen species of *Salmonella*, including a new species, were isolated from symptomless domestic animals and poultry (Table I).

TABLE I

Isolation of species of *Salmonella*

Salmonella sp.	Source*
S. chester	Fowl (1)
S. san-diego	Fowl (1); Duck (1)
S. stanley	Fowl (1); Pig (1)
S. typhimurium	Buffaloe (1)
S. richmond	Fowl (3); Dead embryo (7)
S. aba	Cattle (1)
S. newport	Pig (1)
S. enteritidis var. *jena*	Buffaloe (1)
S. mathura n. sp.	Cattle (1)
S. anatum	Fowl (2); Buffaloe (1); Pig (1); Rat (1)
S. weltevreden	Fowl (10); Buffaloe (4); Goat (2)
S. charity	Cattle (1)
S. hvittingfoss	Fowl (2)
S. magwa	Cattle (2); Buffaloe (1); Goat (1)
S. pomona	Fowl (1); Cattle (2); Buffaloe (1)
S. matopeni	Fowl (1); Cattle (1); Goa (1)
S. champaign	Fowl (2)

* The figures in bracket show the number of isolations.

Description of *S. mathura* n. sp.

S. mathura n.sp. was typed serologically [(9), 46: i: enz 15] by Dr. Edwards (1960) and Dr. Taylor (1960). Dr. Kauffmann (1960) confirmed their findings and also studied the biochemical behaviour of this species. Negative reaction after 30 days in adonitol, rhamnose, salicin, inositol, lactose and sucrose; positive reaction after 1 day in dulcitol, sorbitol, arabinose, xylose, maltose, mannitol, glucose and trehalose; gas production in mannitol and glucose; Stern's glycerolfuchsin broth negative after 8 days; no production of H_2S in ferrous chloride gelatine; gelatine not liquefied; positive reaction in ammonium-glucose and ammonium-citrate; nitrate, +ve; Voges-Proskauer −ve; methyl red, +ve; urea, −ve; d-tartrate, +ve; l-tartrate, +ve; l-tartrate, +ve; sodium citrate, +ve; and mucate, +ve.

Discussion

Thirty-seven species of *Salmonella* have so far been recorded in India from man and animal. *S. aba* from cattle has been recorded for the first time in the present work and according to Edwards (1960) it is rarely found. *S. anatum* from buffaloe and rats, *S. Champaign*, *S. chester* from fowl *S. san-diego* from fowl and duck and *S. stanley* from fowl and pig have been reported for the first time from India. *S. charity*, *S. magwa* and *S. matopeni*, recorded here, according to Edwards (1960) are extremely rare in U.S.A.

Ganguli (1958) has isolated *S. typhimurium* from different tissues in man in India. It is widespread among animals. The strain isolated in the present study belongs to

phage type 12 of Callow (1959). The phage typing was kindly done by Dr. E. S. Anderson, Central Enteric Reference Laboratory and Bureau, London.

Some of the *Salmonella* sp. reported have been encountered in outbreaks of the disease in humans (Freeman, 1953; Ganguli, 1958; Hayes and Freeman, 1945) in India. They may therefore be important in transmitting the disease from animals to humans.

Summary

The occurrence of seventeen *Salmonella* sp. is reported from symptomless domestic animals and poultry in Uttar Pradesh. The description of a new species, *S. mathura* has been given.

Acknowledgment

The authors are indebted to Major L.D. Sachdeva of Armed Forces Medical College, Poona for the help in serotyping Salmonella spp. and Dr. Joan Taylor of Salmonella Reference Laboratory, London, and Dr. P. R. Edwards, Chamblee Georgia, U.S.A. for serotyping of the strains. The authors gratefully acknowledge the kind help of Dr. William B. Cherry, Chamblee Georgia U.S.A. for supplying *Salmonella*-genus-specific o-1 phage, of Dr. J. B. Shrivastav, National Salmonella Centre, Kasauli, Punjab, Dr. F. Kauffmann, International Salmonella & Escherichia Centre, Copenhagen for final confirmation of the new species and Sri C. V. G. Choudary, Principal, Veterinary College, Mathura for providing necessary facilities.

REFERENCES

Callow, B. R. (1959), *J. Hyg.*, **57**: 346.
Cherry, W. B. Davis, B. R., Edwards, P. R. and Hogan, R. B. (1954), *J. Lab. clin. Med.*, **44**: 51.
Edwards, P. R. and Bruner, D. W. (1942), *St. Cir.* 54. United Kentucky Agr. Exptl. Station.
Edwards, P. R. Bruner, D. W. and Moran, A. B. (1948), *J. infect. Dis.*, **83**: 220.
Edwards, P. R. (1960), Personal communication.
Freeman, J. F. (1953), *J. Army Med. Cps.*, **9**: 67.
Ganguli, S. (1958), *Indian med. J. Res.*, **46**: 637.
Hayes, W. and Freeman, J. F. (1945), *Indian J. med. Res.*, **33**: 177.
Kauffmann, F. (1951), *Enterobacteriaceae*, Einar Munksgaard, Copenhagen.
Kauffmann, F. (1960), Personal communication.
Mackie, T. J. and McCartney, J. E. (1956), *Handbook of Practical Bacteriology*, 9th ed., E. & S. Livingstone Ltd. Edinburgh and London.
Pathak, R. C. and Singh, C. M. (1959), *Indian J. Pub. Hlth.*, **3**: 177.
Smith, H. W. and Buxton, A. (1951), *Brit. med. J.* June 30, no. 1478.
Taylor, J. (1960), Personal communication.

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REVIVAL OF NITROGEN-FIXING CAPACITY OF A STRAIN OF *RHIZOBIUM* OF BERSEEM BY SEED INOCULATION IN SOIL

R. B. REWARI

From the Indian Agricultural Research Institute, New Delhi

(Received for publication, December, 1960)

Enhancement of the increased rate of growth in leguminous crops and stimulation of activity of *Rhizobium* resulting in greater fixation of nitrogen, owing to the treatment of the soil with phosphates, have been well established (Truesdell, 1917; Sircar and

Sen, 1940; Poschenrieder, Sammet and Fischer, 1940; Parr and Bose, 1947; Madhok and Dhingra, 1951; Vyas and Desai, 1953). Rogers and Sturkie (1939) observed that the application of phosphates along with inoculation increased nodulation and nitrogen content of hairy vetch. Acharya and Jha (1954) found greater microbial activity in the soil where berseem had been treated with superphosphate. Sen and Bains (1955) noted that the nitrogen content and nodulation of berseem crop responded readily to phosphate application. Rewari and Sen (1958) showed that a strain of *Rhizobium* isolated from the nodules of guar, grown in soil with different phosphate treatments, gave 89% increased nitrogen content of the plants over the control.

The present work deals with the revival of the efficiency of a strain of *Rhizobium* of berseem by seed inoculation in combination with phosphate application to the soil. The efficiency is judged by the increase in yield and nitrogen contents of the plants.

Materials and Methods

Unmanured soil was collected from I.A.R.I. farm from Paddock area. It had not been cultivated. P_2O_5 content of the soil was 0·0014% (29·28 lb./acre) and the nitrogen content was 0·0336%. Fifteen lb. of the soil was put in each pot. Two sets of pot experiments were conducted.

1. With a basal dressing of superphosphate @ 5 mg. P_2O_5/100 g. of soil (100 lb. P_2O_5/acre).

2. Without a basal dressing of superphosphate. The treatments were:

1. Control. 2. KNO_3 control @ 60 lb N/acre. 3. Seeds inoculated with strain A (*Rhizobium* of berseem originally effective but lost its efficiency after being maintained in laboratory for 10 years). 4. Seeds inoculated with strain B (*Rhizobium* of berseem effective and had been maintained in the laboratory for 1 year). Five replications were kept for each treatment. The seeds were sown on 20.11.59. Cuttings were taken on 20.1.60, 15.2.60, 12.3.60 and 19.4.60. Fresh and dry weights were determined. Nitrogen was estimated by Kjeldahl Gunning method.

Results

The data in Table I show that *Rhizobium* strain A of berseem that had lost to great extent the power of fixing nitrogen due to laboratory cultivation, gave less fixation of nitrogen compared with the effective strain B of *Rhizobium* of berseem when inoculated without a basal dressing of superphosphate. When the seeds were inoculated with strain A and B of *Rhizobium* of berseem and sown in soil having a basal dressing of superphosphate, the power of fixing nitrogen of strain A was the same as that of strain B (Table II).

Summary

It has been shown that the nitrogen-fixing capacity of a strain of *Rhizobium* of berseem, that was originally effective and had lost to a great extent its nitrogen-fixing power being in artificial culture, could be revived by the application of phosphate to the soil.

TABLE I

Oven dry weight and nitrogen content of the plants in g. and mg. respectively

Treatment	1st cutting	2nd cutting	3rd cutting	4th cutting	Total of four cuttings	Total N in plants
1. Control	0·100	0·590	1·070	2·660	4·420	115·10
	0·110	0·450	0·570	2·070	3·200	81·54
	0·095	0·550	0·850	0·750	2·245	69·78
	0·065	0·480	0·900	1·180	2·625	73·10
	0·105	0·620	0·790	0·830	2·345	79·45
Average	0·095	0·538	0·836	1·498	2·967	83·78
2. KNO_3 Control	0·075	0·400	0·750	2·080	3·305	98·10
	0·070	0·410	1·400	2·080	3·960	107·55
	0·170	0·925	2·110	3·160	6·365	176·64
	0·100	0·500	1·000	3·330	4·930	120·10
	0·075	0·550	1·950	5·600	8·175	187·70
Average	0·098	0·557	1·442	3·250	5·347	137·10
3. *Rhizobium*	0·105	0·365	0·870	3·200	4·540	119·50
strain A	0·090	0·560	1·250	4·140	6·040	170·81
	0·075	0·470	1·040	2·250	3·835	94·50
	0·075	0·420	0·880	2·760	4·135	104·20
	0·060	0·470	0·620	2·830	3·980	98·07
Average	0·081	0·457	0·932	3·036	4·506	117·41
4. *Rhizobium*	0·080	0·520	0·970	3·860	5·430	133·80
strain B	0·115	0·780	1·410	3·980	6·285	139·02
	0·120	0·660	1·230	3·200	5·210	138·59
	0·110	0·505	0·760	3·540	4·915	125·23
	0·120	0·640	1·820	6·400	8·980	221·27
Average	0·109	0·621	1·238	4·196	6·164	151·58
Yield: Significant at 5% level						
C.D. at 5%—1·913					1 3	2 4
Nitrogen: Significant at 5% level						
C.D. at 5%—45·24			1 3	2 4		

TABLE II

Oven dry weight and nitrogen content of the plants raised with a basal dressing of superphosphate (100 lb. P_2O_5/acre) in g. and mg. respectively

Treatment	1st cutting	2nd cutting	3rd cutting	4th cutting	Total of four cutting	Total N in plants
1. Control	0·430	1·110	0·740	0·670	2·950	84·25
	0·480	1·280	1·060	1·750	4·580	130·81
	0·550	1·635	1·150	1·300	4·635	134·97
	0·540	1·660	1·960	2·800	6·960	179·29
	0·565	1·420	1·400	2·100	5·485	153·58
Average	0·513	1·423	1·262	1·724	4·922	136·58
2. KNO_3 control	0·300	1·130	2·510	1·940	5·880	167·93
	0·355	0·690	1·650	2·550	5·245	152·73
	0·190	0·475	0·570	1·030	2·265	71·03
	0·510	1·210	1·620	3·200	6·540	179·46
	0·615	1·790	1·650	3·300	7·355	220·36
Average	0·394	1·059	1·600	2·404	5·457	158·30
3. *Rhizobium* strain A	0·530	1·300	2·370	7·430	11·630	335·41
	0·990	2·000	3·020	6·910	12·920	332·82
	0·580	1·505	2·320	6·870	11·275	312·54
	0·540	1·705	2·180	4·700	9·125	263·17
	0·570	1·240	2·880	7·660	12·350	328·51
Average	0·642	1·550	2·554	6·714	11·460	314·49
4. *Rhizobium* strain B	0·465	1·240	2·570	5·550	9·825	280·60
	0·615	1·230	2·210	6·650	10·805	284·39
	0·665	1·640	3·720	8·510	14·535	394·77
	0·485	1·000	2·520	6·790	10·795	293·19
	0·465	1·355	2·500	7·230	11·550	303·10
Average	0·539	1·313	2·704	6·946	11·502	311·39

Yield: Significant at 1% level
C.D. at 1%—3·102

1 2 3 4

Nitrogen: Significant at 1% level
C.D. at 1%—79·38

1 2 4 3

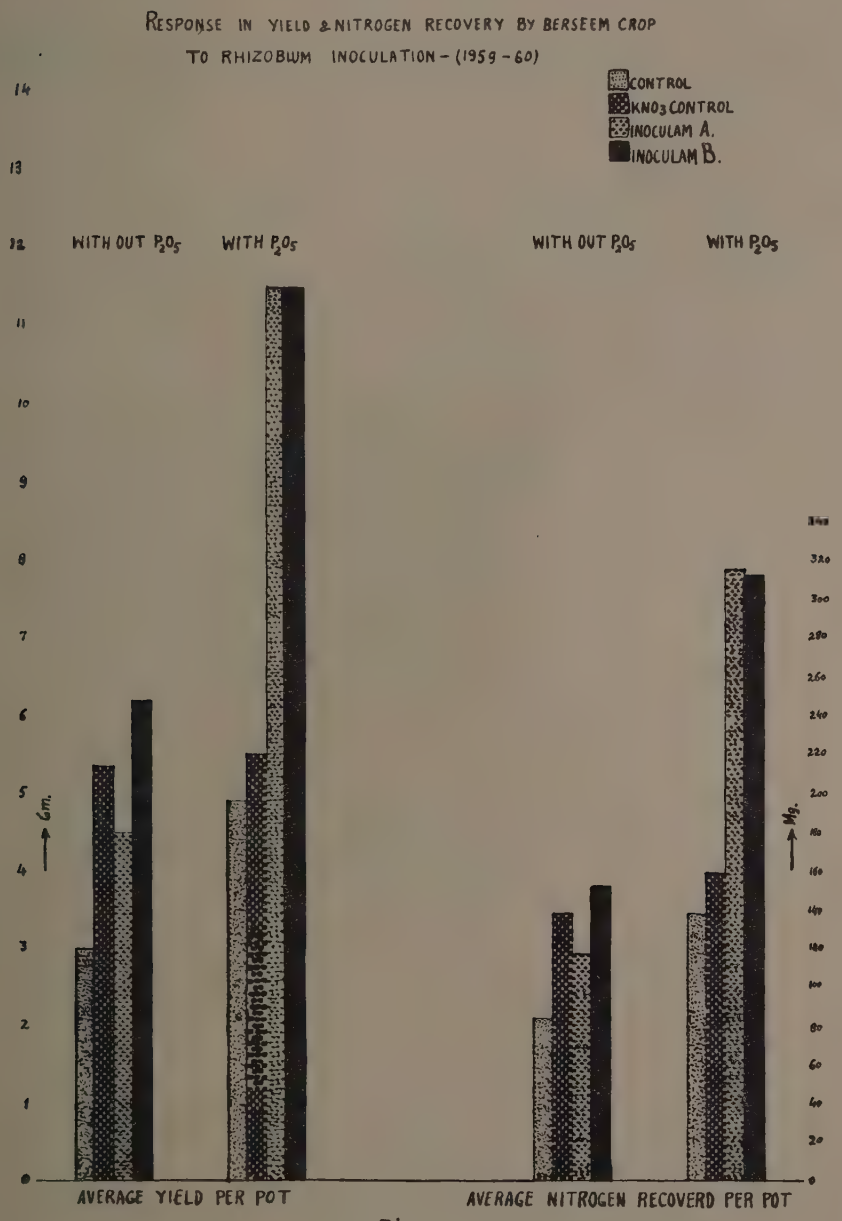

Fig. 1

Revival of nitrogen-fixing capacity of a strain of *Rhizobium* of berseem by seed inoculation in soil.

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REFERENCES

Acharya, C. N. and Jha, J. (1954) *J. Indian Soc. Soil Sci.*, **2**: 21.
Madhok, M. R. and Dhingra, L. R. (1951), *Proc. Indian Sci. Congr.*, 38th, Pt. III, 170.
Parr, C. H. and Bose, R. D. (1947), *Indian Fmg.*, **5**: 201.
Poschenrieder, H., Sammet, K and Fischer, R. (1940), *Zbl. Bakt.*, II, **102**: 388.
Rewari, R. B. and Sen, A. (1958), *Indian Phytopath.*, **11**: 53.
Rogers, T. H. and Sturkie, D. G. (1939), *J. Amer. Soc. Agron.*, **31**: 141.
Sen, and Bains, S. S. (1955), *J. Indian Soc. Soil Sci.*, **3**: 41.
Sircar, S. M. and Sen, N. K. (1940), *Sci & Cult.*, **5**: 634.
Truesdell, H. W. (1917), *Soil Sci.*, **3**: 77.
Vyas, N. D. and Desai, J. R. (1953), *J. Indian Soc. Soil Sci.*, **1**: 41.

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MICROBIOLOGICAL COMPOSITION OF COMMERCIAL LEGUME INOCULANTS

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A legume inoculant is supposed to consist of a pure culture of one effective strain of root nodule bacteria (*Rhizobium*) or a mixture of two or more strains. Therefore, it was considered to be of interest to study the microbiological composition of the commercial legume inoculants, manufactured in New Zealand and imported for sale to farmers in New Zealand, in order to ascertain their quality. The results of these studies are presented in this communication.

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TABLE I

Particulars of inoculants

Trade name	Manufacturer and country of origin	Suitable for inoculation of	Date of expiry of utility	Reference in the text	@% effiiciency on the host tested
Nod-o-gen	Dickinson Co; Chicago, USA	Navy, kidney, string and wax beans.	Oct. 1956	A	113·2—Broadbeans
Do	Do	Vetches, Australian winter peas, Canadian and garden peas	Sept. 1956	B	257·9 —Peas (green feast)
Do	Do	Clovers	—	C	82·0 —White clover
Do	Do	Alfalfa and sweet clover	July. 1956	D	13·83—Lucerne
Inocule	Inocule Lab. Melbourne Victoria E. 10 Australia	Lucerne, barrel clover, and all medics, mellilotes and Bokhara clover	30-7-56	E	74·33—Lucerne
Do	Do	White and red clover strains, cow grass, straw berry, berseem (crimson), Alsike and cluster clovers	30-7-56	F	68·6 —White clover 28·6 —Sub-clover 57·1 —Red clover
Nodulaid Group A	Agril. Labour. Pty. Ltd., Sefton N.S.W. Australia	Lucerne, Bokhara clover, barrel clover and all medics	20-8-56	G	27·1 —Lucerne
Nodulaid Group B	Agril. Labour. Pty. Ltd. Sefton N.S.W. Australia	White, ball strawberry Alsike, hop and wooly clovers, red berseem and Ladino clover	12-5-56	H	80·7 —White clover 52·3 —Red Clover
Nodulaid Group C	Do	Crimson clover and all strains of sub-terranean clover	1-6-56	I	68·1 —White clover 46·4 —Red clover
Biolab.	Biolab. Auckland New Zealand	Lucerne	—	J	56·9 —Lucerne
Do	Do	Clovers	—	K	74·8 —Red clover 87·9 —White clover

@% Efficiency represents increase over uninoculated control in terms of total protein.

TABLE II

Count of Rhizobia *in* 100,000 *per g. of the inoculants on dry weight basis*

Month	A	B	C	D	E	F	G	H	J	J	K
March	3·79	2·89	5·00	8·77	10·81	3·44	—	—	—	—	—
April	2·53	1·45	7·50	11·43	17·57	2·30	38·59	—	—	—	—
May	2·53	2·89	10·00	14·29	14·87	2·30	36·84	—	10·29	8·06	10·00
June	2·53	4·34	10·00	18·57	21·62	5·74	29·82	12·07	8·82	9·67	8·57
July	2·53	2·89	7·50	22·86	16·22	4·59	35·09	8·62	8·82	9·67	7·14
August	1·26	1·45	1·25	7·14	13·52	1·14	21·05	5·17	4·51	9·67	5·71
September	—	1·45	1·25	5·71	10·81	—	17·54	3·44	2·94	3·22	5·71
'k'	0·151	0·173	0·349	0·278	0·098	0·349	0·091	0·186	0·173	0·143	0·063
1/'k'	6·6	5·79	2·87	2·33	10·17	2·86	10·95	5·38	5·78	6·97	15·97
Moisture % initial	21·43	28·59	20·34	30·83	26·48	13·41	43·67	41·73	32·68	38·23	30·56
Moisture % final	21·87	29·25	20·94	30·98	27·51	13·52	42·90	41·56	33·03	37·95	30·85

TABLE III

Count of identified bacteria, fungi and actinomyces in millions per g. of the inoculants on dry weight basis

	A	B	C	D	E	F	G	H	I	J	K
Bacteria											
Bacillus cereus	—	1·45	—	1·74	—	—	29·82	24·14	2·94	3·23	8·57
Bacillus megaterium	—	—	—	8·57	25·68	—	5·26	5·17	—	—	—
Streptomyces sp.	—	—	1·25	94·28	56·75	1·15	—	—	16·17	1·61	8·57
Arthrobacter and Agrobacterium sp.	—	201·5	147·15	382·8	164·5	298·8	5·26	3·45	45·59	122·6	11·43
Fungi											
Pencillium sp.	1·26	1·45	1·25	4·29	1·35	1·15	3·51	—	20·59	29·03	38·58
Botrytis sp.	—	1·45	—	4·29	—	—	1·75	—	14·71	24·19	25·71
Trichothecium sp.	—	—	—	2·86	1·35	1·15	3·51	—	17·65	27·42	30·00
Actinomyces											
Streptomyces lavendulae	30·38	4·35	59·99	37·15	27·03	82·75	—	—	41·18	41·94	44·29

Materials and Methods

Particulars of eleven peat-based legume inoculants imported and manufactured in New Zealand, used in the work, are presented in Table I.

One g. of the inoculant was serially diluted in sterile water and one ml. of it at 10^5 dilution was plated in duplicate on yeast mannitol agar (Fred and Waksman, 1928). The count of *Rhizobia* was taken after ten days of incubation at 25°C and the mean count per g. of the inoculant was determined on dry weight basis. The loss in viability with time was expressed as 'K', the average logarithmic decline per month and its reciprocal gives the time in months for a ten-fold reduction of the viable count (Vincent, 1958).

For the count of bacteria, other than *Rhizobia*, the inoculant was serially diluted in sterile water and one ml. of it at 10^2 dilution was plated in duplicate on nutrient gelatin and incubated at 18° C. The types of bacteria were identified according to Conn (1948).

The actinomyces was counted from 10^6 dilution of the inoculant on glucose asparagine agar (Waksman, 1950) after 7 days incubation at 25° C, by the method described above. The dominant types of actinomyces were isolated in pure culture and identified according to Riddel (1950).

The procedure for the count of fungi was the same as that for the actinomyces except that potato-dextrose-agar (Waksman, 1950) was used as the medium.

The moisture content of the inoculants was determined at the time of storage in a refrigerator and at the end of seven months.

Results

The variations in the viable counts of *Rhizobia* in the inoculants at monthly intervals, during a period of seven months of their storage in a refrigerator, and the moisture contents of the inoculants are presented in Table II. During the period of storage of the inoculants, there was practically no loss of moisture. A decrease in the number of viable cells of *Rhizobia* commenced generally after five months, by which time the majority of the inoculants passed the date of guarantee of their utility. The 'K' values (see materials and methods) ranged between 0·063 to 0·349 indicating that viability was maintained at a high level. The rate of decrease, as shown by the reciprocal of 'K' values, showed wide variations from 2·33 to 15·97.

In Table III the number of bacteria, other than *Rhizobia*, actinomyces and fungi in the inoculants are presented. In general, the inoculants contained a large population of bacteria followed by the actinomyces and the number of fungi was the least. The inoculant A did not contain any bacteria and H was free from both actinomyces and fungi. Among the species of bacteria, *Arthrobacter and Agrobacterium* sp. were present in much higher number in most of the inoculants. *Streptomyces lavendulae* occurred in nearly all the inoculants in fairly large number.

The number of fungi was low in all the inoculants except I, J and K.

Discussion

Vincent (1958) has shown a relationship between the death of *Rhizobia* and the rate at which there is a loss of water from the inoculants. In the present work it has

been shown that during seven months of storage in a refrigerator, the peat-based legume inoculants did not lose any appreciable amount of water. The decrease in the viable counts of *Rhizobia* commenced only after five months, by which time the majority of the inoculants passed the date of guarantee of their utility, as declared by the manufacturers. Therefore, all the inoculants may be regarded as satisfactory. The rate of decrease in the *Rhizobia* numbers, as shown by the reciprocal values of 'K', varied within wide limits in the inoculants. This may be due to the varying conditions under which the inoculants were manufactured. The reciprocal of 'K' values might be a better criterion for judging the quality of the inoculants. The viability of *Rhizobia* depends also on the nature of the substrate (sterilized or unsterilized), initial number of *Rhizobia*, percentage moisture and aeration (Sankaram, 1959). The degree of contamination by microorganisms, other than *Rhizobia*, is another criteria to evaluate the quality of inoculants (Sankaram, 1958). Vincent (1954) suggested that "contaminants shall not exceed the number of *Rhizobia*." None of the inoculants under study satisfy this criteria. Leonard (1944) and others observed that the presence of contaminating organisms does not seem to affect the efficiency of *Rhizobia*. None of the contaminating bacteria, actinomyces and fungi isolated from the inoculants used were antagonistic to *Rhizobia* (Purchase, 1959). This may be one of the reasons why the inoculants, when tested on their related hosts, were found to be efficient in nitrogen-fixation. The inoculants manufactured in New Zealand were as good as the imported ones.

Summary

Monthly counts, up to seven months, of *Rhizobia*, bacteria other than *Rhizobia*, actinomyces and fungi were made by plating technique from eleven peat-based legume inoculants, imported and manufactured in New Zealand, kept in a refrigerator. There was no appreciable loss of water from the inoculants during this period. The decrease in the viable counts of *Rhizobia* commenced only after five months, by which time the majority of the inoculants had passed the date of guarantee of their activity given by the manufacturers. None of the contaminating bacteria, actinomyces and fungi isolated were antagonistic to *Rhizobia*.

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REFERENCES

Conn, H. J. (1948), *Bact. Rev.*, **12**: 257.

Fred, E. B. and Waksman, S. A. (1928), *Laboratory Manual of General Microbiology*, McGraw-Hill Book Co., New York.

Leonard, L. T. (1944), *Method of testing legume bacteria cultures and results of commercial inoculants in 1943*, U.S.D.A. Circular No. 703.
Purchase, H. F. (1959), Private communication.
Riddel, R. W. (1950), *Mycologia*, **42**: 265.
Sankaram, A. (1958), *Andhra Agric. J.*, **5**: 176.
Sankaram, A. (1959), (in press).
Vincent, J. M. (1954), *J. Australian Inst. Agric. Sci.*, **20**: 247.
Vincent, J. M. (1958), *Survival of the root nodule bacteria*, Butterworths Sci. Pub., London.
Waksman, S. A. (1950), *The Actinomycetes*, Chronica Botanica, Waltham, Mass, U.S.A.

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INCIDENCE OF ENTEROVIRUSES AMONG HEALTHY INDIVIDUALS OF BOMBAY

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The Coxsackie and ECHO viruses share many properties with the poliovirus and, therefore, they have been considered as members of a single family of enteroviruses (Committee, 1957). At present the human enterovirus family includes three types of poliovirus, 30 types of Coxsackie virus (24 in group A, 6 in Group B) and 28 types of ECHO virus. New types are still being added to the ECHO group. Coxsackie and ECHO group of viruses, although associated with disease conditions, have also been isolated from healthy individuals (Ramos Alvarez and Sabin, 1954; Gelfand, Fox and Leblanc, 1957; Ormsbee and Melnick, 1957 and others). This has made it difficult to assess their aetiological role.

Work done on enteroviruses in India has been reviewed by Bhagwat and Gharpure (1961). The present work deals with the isolation and identification of enteroviruses from healthy human subjects.

Materials and Methods

Two hundred and thirtytwo samples of stool were collected from apparently healthy individuals of Bombay, belonging to different age groups, to both sexes (126 males and 106 females), during winter and monsoon months as shown in Table I.

Homogenous 10% suspensions of stools were made in Hanks' balanced salt solution and were inoculated in rhesus monkey kidney tissue cultures, prepared by Bodian's (1956) method. The kidney tissue was broken down to single cells by the action of 0·2% trypsin at 22°C for one hr. and at 4°C for 18 hr. The cytopathogenic effect indicated the

TABLE I

Age-wise and month-wise collection of stool samples

Age in years	No. of stools collected in different months									
	Winter					Late summer or monsoon				
	November 1958	December 1958	January 1959	February 1959	Total	July 1959	August 1959	September 1959	Total	
0—1	4	4	0	18	26	3	8	4	15	41
1—4	6	11	1	32	50	1	18	6	25	75
4—9	10	9	1	7	27	1	4	6	11	38
10 and above	8	48	22	0	78	0	0	0	0	78
Total	28	72	24	57	181	5	30	16	51	232

presence of virus. When no cytopathogenic effect was observed in 7 days of inoculation, the culture fluids were passaged again to determine the presence of virus.

With a view to isolating group A Coxsackie viruses (types 1 to 8 and 10 to 19), which usually fail to grow in tissue culture, stool suspensions were inoculated in infant mice, less than 48 hr. old, by the subcutaneous route (0·02 ml./mouse). Stools were sampled at random from different municipal wards of Bombay. Some times 4 to 5 stools were pooled before inoculation. The animals were observed for a period of 15 days for the appearance of tremors, wrist-drop, paralysis etc.

Once a virus strain was established, its 50% infectivity in tissue culture ($TCID_{50}$) was calculated by the method of Reed and Muench (1938). 100 $TCID_{50}$ of each cytopathogenic agent was typed against three types of polio antisera. The non-polio viruses were tested against pools of antisera made up as follows:

(*i*) Coxsackie types A_9 and B_1 to B_5 (Coxsackie antiserum pool)
(*ii*) ECHO types 2, 3, 5 and 6 (ECHO pool 1 antiserum)
(*iii*) ECHO types 7 to 11 (,, ,, 2 ,,)
(*iv*) ECHO types 1, 12 and 13 (,, ,, 3 ,,)

Once a cytopathogenic agent showed neutralization with an antiserum pool, it was tested against individual antisera in that pool.

Plaque morphology of all the 32 cytopathogenic agents was studied. The bottle cultures were prepared by the method of Hsiung and Melnick (1957). Plaques were counted every day and their first appearances, size and other morphological characters observed.

The poliovirus strains were studied for their pathogenicity in monkeys. Rhesus monkeys were inoculated intra-cerebrally (0·5 ml. / monkey) with the poliovirus strains and the animals were observed for a period of 30 days. Rise in body temperature and the appearance of tremors and paralysis were looked for.

The non-polio virus strains were tested for their pathogenicity in infant mice, less than 48 hr. old. Stool suspensions as well as the infective tissue culture fluids were inoculated subcutaneously (0·02 ml. / mouse). The animals were observed for 15 days as described earlier. Mice showing signs of illness were divided into two batches, one batch was kept for the study of virus and the second for histological studies.

Fertile eggs, 9 to 11 days old, were inoculated with a virus strain (0·15 ml. / embryo) by the allantoic route. Ten strains (one poliovirus type 2, one Coxsackie A_9, one Coxsackie B_4 three ECHO viruses and four unidentified ones) were employed in this work. At least three passages were made in eggs by the same route. The harvested allantoic fluids were passaged in tissue culture and the presence of cytopathogenic effect was determined.

Results

Isolation and identification: Of the 232 stool specimens inoculated in tissue culture, 32 cytopathogenic agents were isolated, at an isolation rate of 13·8%. Of these, 9 were identified as polioviruses (one type 1, two type 2 and six type 3), seven were neutralized by the Coxsackie pool, three by ECHO pool 1, three by ECHO pool 2 and four by ECHO pool 3 antisera. Six strains did not show neutralization with any antiserum pool

(Table III). By testing against individual antisera, five were identified as Coxsackie A_9, two as Coxsackie B_4, three as ECHO type 3, three as ECHO type 11 and four as ECHO type 1. Out of the 200 stools negative for virus in tissue culture, 88 were tested in infant mice but no virus could be isolated.

TABLE II

Results of egg inoculation with different viruses

Strain No. (KMM)	Serological types	Serial passage in chick embryo						
		EP* 1	EP 2	EP 3	EP 4	EP 5	EP 6	EP 7
84	ECHO—type 1	++	o	o	o	o		
P/7	ECHO—type 1	o	o	o				
231	ECHO—type 3	o	o	o				
60	ECHO—type 11	++	+	o	o	o		
168	ECHO (unidentified)	o	o	o				
P/10	ECHO [illegible]	o	o	o				
P/9	Coxsackie type A_9	+++	++	—	o	o		
210	Coxsackie type B_4	++	+	o	o	o		
P/3	Polio type 1	−+−	−+−	+++	+++	+++	+++	+++

*EP = Egg passage; + = degree of infectivity in tissue culture.

In stool specimens showing virus, poliovirus was present in 28%, Coxsackie in 22% and ECHO in 31%; 19% cytopathogenic agents could not be identified. Of the polioviruses isolated, type 1 constituted only 11·1%. Duncan, Peach and Rhodes, (1954) and Gharpure, Swaminathan and Dave (1955) have reported a high percentage of this type of poliovirus among polio patients. 66·6% of the polioviruses isolated by us were type 3. The two group B Coxsackie virus isolated were from children without any signs of illness. Rhodes and Beale (1957) and Paul, Gupta and Gupta (1959) have stated that the mere isolation of group B Coxsackie virus is of aetiological importance. All the five Coxsackie A_9 virus strains were isolated from children residing in Cusrow Baug in Ward A. This indicated that group A Coxsackie viruses existed in high proportion at least in this locality. No other type of Coxsackie A virus was isolated by inoculating infant mice. This finding does not agree with those of Banker (1952) and Pathak (1958) who have shown the prevalence of group A Coxsackie viruses, especially type A4. This may be due to the fact that stool specimens used by us were diluted by pooling 4-5 samples and also because of their storage in frozen condition at —20°C for 6 to 14 months before inoculating them in the suckling mice. The three types of ECHO virus (types 1, 3 and 11) encountered in the present study have also been isolated frequently by other workers investigating the enteric viral flora of healthy individuals (Ramos Alvarez and Sabin, 1954; Ormsbee and Melnick, 1957; Melnick and Agren, 1952).

TABLE III

Age-wise distribution of enterovirus

Age Group (years)	Samples collected	Samples positive	% positive	Poliovirus types			Coxsackie types	virus	ECHO virus types			Unidentified cyto-pathogenic agents
				1	2	3	A 9	B-4	1	3	11	
0—1	41	1	2·4	0	0	0	0	0	0	0	0	1
1—4	75	21	28·0	0	2	4	5	1	4	1	1	3
4—9	38	6	15·8	1	0	1	0	1	0	1	2	0
10 and above	78	4	5·1	0	0	1	0	0	0	1	0	2
Total	232	32	13·8	1	2	6	5	2	4	3	3	6

Age incidence. As shown in Table III, the incidence of enteroviruses in general, decreased with increase in age. These results are in agreement with the findings of Ramos Alvarez and Sabin (1954) and Polio Research Unit, Bombay (1955-59 data).

Sex incidence. None of the major surveys has reported a significant difference in the sex-incidence for enteroviruses. Similar results of incidence were obtained from both males (13·5%) and females (14·1%).

Seasonal incidence. The enteroviruses were excreted about three times more frequently in the monsoon months (29·4%) than in the winter months (9·4%). Longitudinal studies of several households in West Virginia (Ormsbee and Melnick, 1957) and in Phoenix, Arizona (Melnick, 1958), have shown a similar pattern of enteric virus excretion. A similar type of seasonal incidence was observed by the Polio Research Unit, Bombay, the peak incidence being in months of July, August and September (Gharpure, unpublished).

Fig. 1. Plaques produced by a polio viruses strain in monkey kidney cultures.

Fig. 2. Plaques produced by a Coxsackie type A9 strain in monkey kidney cultures.

Fig. 3. Plaques produced by an ECHO type 3 strain in monkey kidney cultures.

Plaque morphology. The poliovirus strains produced large circular plaques with sharp uniform boundaries on the 2nd or 3rd day. The plaques increased in size upto 6 days when they were 2 to 2·5 cm. in diameter. The Coxsackie virus strains produced circular plaques resembling polio plaques but smaller and appeared 3 to 5 days after inoculation. Plaques produced by type A_9 Coxsackie viruses were found to be larger (about 1·0 cm.) than those produced by type B_4 (about 0·6 cm.). The ECHO virus plaques were the smallest (0·2 to 0·5 cm.) and were irregular in shape with diffused margins. They first appeared on the 4th, 5th or 6th day (*Fig. 1*). The 6 unidentified strains produced plaques similar to those produced by ECHO virus strains.

Animal infectivity. Of the nine polioviruses inoculated in rhesus monkeys, seven were found to be paralytogenic and two (one type 2 and one type 3) non-paralytogenic. Among the 23 non-polio virus strains, inoculated in infant mice, seven were pathogenic, five induced frank paralysis and death in the new born mice in 4 to 5 days and two only paralysed (but not killed) the baby mice in about nine days. The first five strains were Coxsackie A_9 and the last two were Coxsackie B_4 serologically. Histologically, the Coxsackie A_9 strains caused extensive degeneration of the skeletal muscles, whereas the Coxsackie B_4 caused focal lesions in brain, in skeletal muscles and fat pads.

Egg infectivity. Reports on successful propagation of enteroviruses in eggs have been few and far between. Cabasso, Stebbins, Dutcher, Moyer and Cox (1952) and Roca Garcia, Moyer and Cox (1952) cultivated type 2 poliovirus in eggs by the allantoic and yolk sac routes respectively. Godenne and Gurnen (1952) propagated a Coxsackie virus in eggs.

The viruses isolated in tissue culture were inoculated in chick embryos and the results are presented in Table II. $P/_{23}$ strain of poliovirus (type 2) could be successfully passaged, upto the 7th passage, in chick embryo. The infectivity of the virus was the same in all the passages, showing that it has been successfully adapted to the eggs. The 1st, 4th and 7th passaged allantoic fluids gave the same titres of the virus.

Summary

Out of 232 stool specimens collected from apparently healthy individuals of Bombay, 32 cytopathogenic agents were isolated in tissue culture, an incidence rate of 13·8%. Out of these 9 were polioviruses (1 type 1, 2 type 2 and 6 type 3), 7 Coxsackie viruses (5 type A_9 ane 2 type B_4) and 10 ECHO viruses (4 type 1, 3 type 3 and 3 type 11); 6 were not identified. Of the 9 polioviruses inoculated in rhesus monkeys, 7 were paralytogenic and 2 were non-paralytogenic. Seven out of 23 were found to infect infant mice. One strain of poliovirus (type 2) could be successfully adapted to chick embryos.

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REFERENCES

Banker, D.D. (1952), *Indian J. med. Sci.*, **6**: 733.
Bhagwat, S.M. and Gharpure, P.V. (1962), *Jour. J. J. Gr. Hosptls.*, **6**:: 27.
Bodian, D. (1956), *Virology*, **2**: 575.
Cabasso, V. J., Stebbin, M.R., Dutcher, R.M., Moyer, A.W. and Cox, H.R. (1952), *Proc. Soc. exp. Biol. Med.*, **81**: 525.
Committee on Enteroviruses (1957), *Amer. J. Pub. Hlth.*, **47**: 1556.
Duncan, D., Peach, A.M. and Rhodes, A.J. (1954), *Canad. J. Pub. Hlth.*, **45**: 396.
Gelfand, H.M., Fox, J.P., and Leblanc, D.R. (1957), *Amer. J. trop. Med.*, **6**: 521.
Gharpure, P.V., Swaminathan, C.S. and Dave, K.H. (1955), *Child Hlth.*, **5**: 125.
Gharpure, P.V. *et al.* (Unpublished), *Ten years' report of Polio Research Unit*, Bombay, Indian Council of Medical Research.
Gifford, R. and Dalldorf, G. (1951), *Amer. J. Path.*, **27**: 1047.
Godenne, M.O. and Curnen, E.C. (1952), *Proc. Soc. exp. Biol. Med.*, **81**: 81.
Hsiung, G.D. and Melnick, J.L. (1957) *J. Immunol.*, **78**: 128.
Melnick, J.L. (1958), *Fourth International Poliomyelitis Conference*, Philadelphia, Lippincott, p. 187.
Melnick, J.L. and Agren, K. (1952), *Proc. Soc. exp. Biol. Med.*, **81**: 621.
Ormsbee, R.A. and Bell, E.J. (1957), *Amer. J. Pub. Hlth.*, **47**: 1405.
Ormsbee, R.A. and Melnick, J.L. (1957), *J. Immunol.*, **79**: 384.
Pathak, S.M. (1958), *Thesis for the Ph. D. degree*, Faculty of Science, University of Bombay.
Paul, S., Gupta, N.P. and Gupta, S.P., (1959), *J. Indian. med. Ass.*, **32**: 312.
Ramos Alvarez, M. and Sabin, A.B. (1954), *Proc. Soc. exp. Biol. Med.*, **87**: 655.
Reed, L.J. and Muench, H. (1938), *Amer. J. Hyg.*, **27**: 493.
Rhodes, A.J. and Beale, A.J. (1957), *Ann. N. Y. Acad. Sci.*, **67**: 212.
Roca Garcia, M., Moyer, A.W. and Cox, H.R. (1952), *Proc. Soc. exp. Biol. Med.*, **81**: 519.

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TRANSAMINASE IN *AZOTOBACTER* SP.

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The occurrence and general importance of transaminases have been discussed since the discovery of these enzymes by Braunstein and Kritzman (1937 a, b). These enzymes catalyse the formation of different amino acids during the nitrogen-fixation process. The presence of transaminases in most types of living cells suggests that they play an important role in nitrogen metabolism. In view of the fact that the *Azotobacter* sp. can

fix atmospheric nitrogen and thus must be capable of extremely rapid protein synthesis, it seemed important to study the transamination reaction in this species. In this paper experiments are reported which establish the presence of a potent transaminase system in *Azotobacter vinelandii* which catalyses the following reaction: α-ketoglutaric acid + aspartic acid → glutamic acid + oxalacetic acid.

Materials and Methods

The culture of *A. vinelandii* was obtained from the National Type Culture Collection, Poona. Three days old culture of bacteria, growing in Burk's nitrogen-free agar medium was washed with M/200 phosphate buffer (*p*H 7·5) twice in a centrifuge. Finally the bacterial cells were suspended in a known volume of M/200 phosphate buffer (*p*H 7·5).

The reaction mixture for studying the transamination reactions was prepared with the known volumes of M/5 phosphate buffer (*p*H 8·0), M/10 α-keto acid (neutralised), M/10 α-amino acid (neutralised), bacterial suspension and distilled water. The transamination reactions were carried out in Thunberg tubes under anaerobic condition. The activity was measured in terms of glutamic acid-N formed.

Results

Transamination reaction between sodium pyruvate and aspartic or glutamic acids was found to be very low and could not be measured quantitatively. Experiments were conducted to find out the effect of time of reaction on the aspartic-glutamic transaminase activity and this gave a linear relationship (Table I). The transaminase activity was also measured at different *p*H values and the optimum *p*H value for the aspartic-glutamic system was 8·0 (Table II).

TABLE I

Effect of incubation time on transamination with Azotobacter vinelandii

Time of incubation min.	Total glutamic acid-N in μg./mg. of dry bacteria	Glutamic acid-N in μg./mg. of dry bacteria produced by transamination
30	1·67	1·25
60	3·22	2·80
120	6·32	5·90

*p*H 8·0; temperature 37°C; bacterial weight 5·5 mg./ml.

Reaction mixture. M/5 phosphate buffer (*p*H 8·0) 0·5 ml.; M/10 α-ketoglutarate 0·2 ml.; M/10 L-aspartate 0·2 ml.; bacterial suspension 1·0 ml.; and distilled water.

TABLE II

Effect of pH *on transamination with* Azotobacter vinelandii

pH	Total glutamic acid-N in g./μg. of dry bacteria	Glutamic acid-N in g./μg. of dry bacteria produced by transamination
6·0	1·40	0·93
6·5	1·55	1·08
7·3	2·16	1·69
8·0	5·81	5·34

Time of reaction 2·0 hr.; temperature 37°C; bacterial wt. 7·0 mg./ml.

Reaction mixture. M/5 phosphate buffer (*p*H 6·0) 0·5 ml.; M/5 phosphate buffer (*p*H 6·5) 0·5 ml.; M/5 phosphate buffer (*p*H 7·3) 0·5 ml.; M/5 phosphate buffer (*p*H 8·0) 0·5 ml.; M/10 α-ketoglutarate 0·2 ml.; M/10 L-aspartate 0·2 ml.; bacterial suspension 1·0 ml.; and distilled water.

The transamination reaction at different concentration of bacterial cells showed increased activity with increased bacterial concentration (Table III).

TABLE III

Effect of bacterial concentration on transamination with Azotobacter vinelandii

Weight of bacteria (dry basis)	Glutamic acid-N in μg.
1·25	4·73
2·50	9·46
3·75	14·19
5·00	18·92

Reaction time 2·0 hr.; *p*H 8·0; temperature 37°C

Reaction mixture. M/5 phosphate buffer (*p*H 8·0) 0·5 ml.; M/10 α-ketoglutarate 0·2 ml.; M/10 L-aspartate 0.2 ml.; and distilled water.

Summary

Transaminase activity was studied in *Azotobacter vinelandii*. The aspartic-glutamic transaminase system, which catalyses the reaction (α-ketoglutaric acid + aspartic acid→

glutamic acid + oxalacetic acid), was found to be present in this organism. Transamination reaction between sodium pyruvate and aspartic or glutamic acid was found to be very slow. Transaminase activity increased with the increased time of incubation up to 2 hr. The optimum *p*H value for the aspartic-glutamic system was 8·0. With the increase of bacterial concentration, the activity was also increased.

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I am deeply indebted to Dr. N. B. Das, the Biochemist, for his guidance throughout the course of the investigation.

REFERENCES

Braunstein, A.E. and Kritzman, M.G. (1937a), *Enzymologia*, **2**: 129.
Braunstein, A.E. and Kritzman, M.G. (1937b), *Nature*, London, **140**: 503.
Lichstein, H.C. and Cohen, P.P. (1945), *J. biol. Chem.*, **157**: 85.

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LETTER TO THE EDITOR

MODE OF ACTION OF THE POLYENE ANTIBIOTIC, NYSTATIN, ON *LEISHMANIA DONOVANI*

The high leishmanicidal activity of the antifungal antibiotic nystatin in both *in vivo* and *in vitro* tests has already been reported from our laboratory (Ghosh *et al.*, 1960 1961). In a series of further studies we have succeeded in elucidating some of the aspects of mode of action of this antibiotic on *L. donovani*. Some of these results obtained are

being briefly described in the present communication. Detailed reports of the present investigation have already been communicated and will be shortly published (Ghosh and Chatterjee 1961).

The high metabolic inhibition of *L. donovani* by nystatin in the presence of different utilizable substrates under both aerobic and anaerobic conditions of incubation (Ghosh *et al.*; 1960) indicated the non-specific nature of its inhibition. If the action of the drug be on anyparticular enzyme, inhibition of that enzyme must explain the metabolic derangement of the organism under such diverse conditions of incubation. With this idea, cell-free extracts of *L. donovani* were prepared and the action of nystatin on some of the key enzyme systems like hexokinase, glucose-6-phosphate dehydrogenase, isocitric dehydrogenase, transaminase, ATPase and cytochrome oxidase were studied in this cell-free system. Details regarding preparation of cell-free extracts and assay conditions for these enzymes have been published elsewhere (Chatterjee, 1959). The results were found to be entirely in the negative and thus though nystatin has been reported to inhibit enzyme activity in fungal systems (Bradley, 1958), the metabolic inhibition observed in *L. donovani* must be due to causes other than enzyme inhibition.

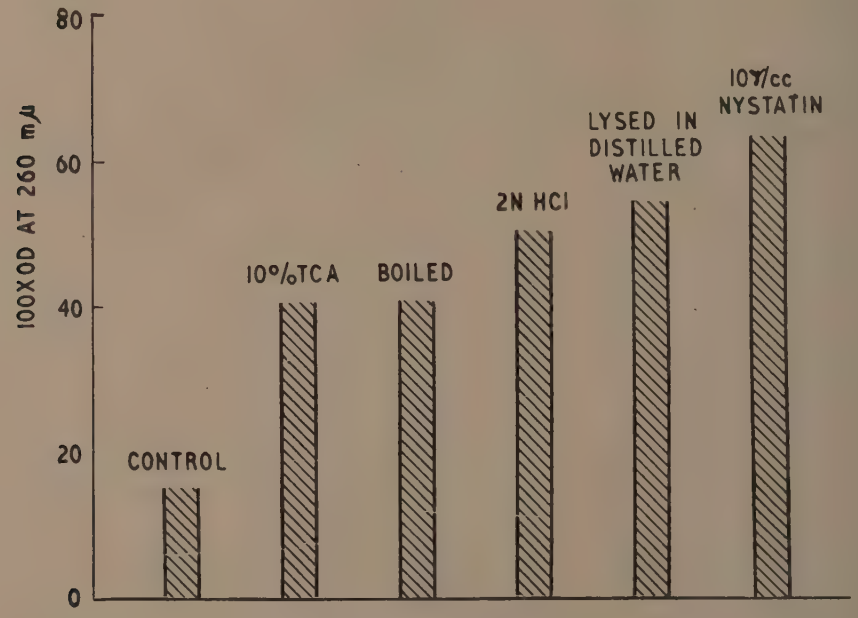

Fig. 1.

As the next logical step, we investigated whether nystatin directly damaged any vital constituent of the cell at the surface level. Since any effect of nystatin on the cell surface of *L. donovani* would result in a leakage of intracellular constituents, the rate and extent of the release of these constituents from the cells in presence of nystatin was

examined. The results are shown in Fig. 1. From the Fig. 1, it may be seen that nystatin causes a release of 260 mμ absorbing materials from the cells whose amounts were comparable to those when the cells were subjected to treatments known to release their free metabolic pool to the incubation medium e.g., boiling the cell suspension or extraction with HCl or TCA. The rate of this release was found to be very rapid and the

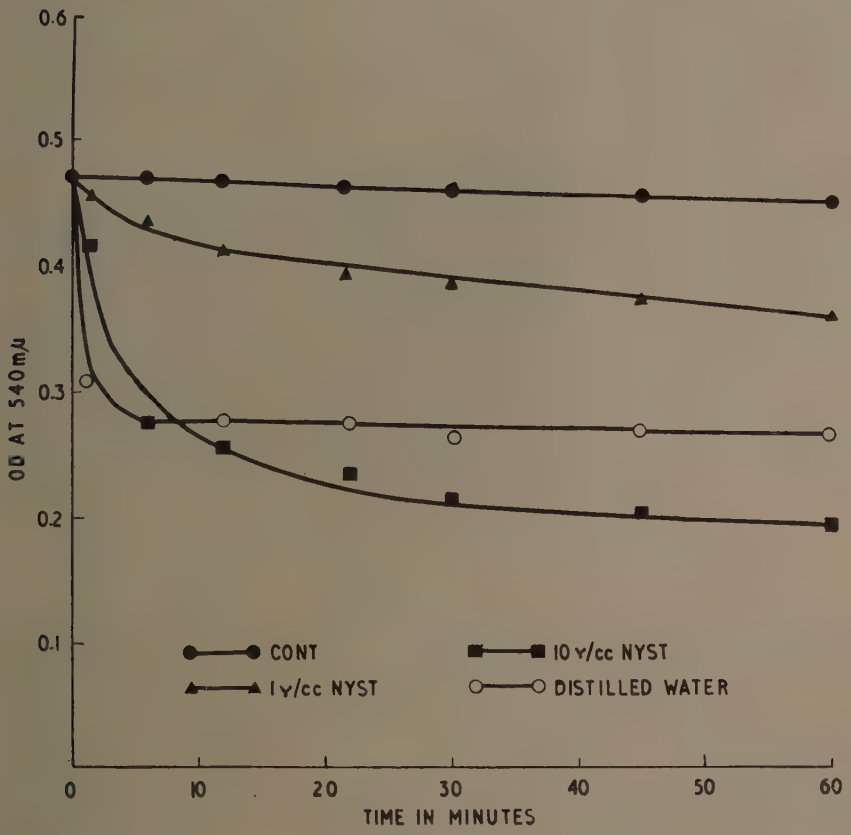

Fig. 2.

major part of the release was completed within the first thirty minutes of incubation with nystatin, and hence autolytic breakdown under these conditions could be ruled out. Furthermore, the optimum conditions for the metabolic inhibition by nystatin (Ghosh *et al.*; 1960) and the release of the intracellular constituents were found to be identical. Apart from the 260 mμ absorbing material, the free amino acid pool was also found to be released

quantitatively from the cells on incubation with nystatin and the kinetics of this release were found to be the same as that for the release of 260 mμ absorbing materials. Besides these low molecular weight substance, TCA precipitable high molecular weight substances were also released from the cells of *L. donovani* on incubation with nystatin and these were identified as biuret positive substances and nucleic acids. In bacteria the cell wall and the cell membrane normally act as barriers for the high molecular weight and low molecular solutes respectively (Mitchell and Moyle, 1956) and compounds which disorganise the cell membrane cause a leakage of only the low molecular weight solutes (Salton, 1955). From our observations it appears that in this hemoflagellate the cell wall may be absent and the plasma membrane acts here as the sole osmotic barrier. Our observations on the absence of lysozyme sensitivity, diaminopimelic acid and muramic acid (both specific constituents of cell wall), inability of the cells to withstand changes in osmotic pressure, absence of any plamolysis etc., all indicate that the cell wall, as found in bacteria, might not be present in *L. donovani*.

From the above results it appeared that nystatin might cause an osmotic imbalance in the cells by damaging the cell membrane resulting in the leakage of intracellular constituents. If this be the case, the cells must ultimately undergo lysis and the results of our experiments on the cellular lysis by nystatin is shown in Fig. 2. From the Fig. 2 it will be seen that there was a rapid lysis of the cells of *L. donovani* by nystatin and also the rate of this lysis could explain the metabolic lesions. The extent of this lysis may be seen to be the same as caused by suspension of the cells of *L. donovani* in distilled water. That nystatin caused an immediate breach in the osmotic barrier of *L. donovani* was also shown by the fact that trypsin and lipase, while without any effect on the normal cells, were highly active when added to a nystatin-treated cell suspension. A similar behaviour of lipase on an osmotic shockate of protoplasts in bacteria has been reported (spiegelman *et al.*; 1958). It might be noted here that Salton, the pioneering worker on bacterial cell walls, regards the phenomenon of lysis of microbial cells, as in the present case, to be the clearest example of a disorganisation at the surface level (Salton, 1955).

As nystatin-treated cell suspensions resembled in some ways the protoplast system in bacteria, attempts were made to stabilize the nystatin-treated cell suspensions by increasing the osmotic pressure of the incubation medium. It was observed that solutes like sucrose, (0.2*M*) and $MgCl_2$ (0.1*M*) could stabilize nystatin treated cell suspensions whereas glycerol had no such stabilizing action. The permeability characteristics of *L. donovani* with respect to these solutes was examined by Mitchell's technique (Mitchell and Moyle, 1956) and it was found that while $MgCl_2$ and sucrose were impermeable to *L. donovani*, glycerol was freely permeable to the cells. The absorption of nystatin must have been prevented in the presence of high concentrations of these impermeable solutes, for when the nystatin-treated cell suspension containing sucrose was centrifuged off, the cells behaved as normal cells when resuspended in normal saline.

We have fractionated the nystatin-treated cell suspension by density gradient centrifugation in sucrose. Three discrete layers of cellular components were thus obtained along with an opalescent supernate. All these layers were spun down, washed free of sucrose by phosphate buffer and then examined by phase contrast and dark ground microscopy. The first layer from the top, which was yellowish in appearance and appeared to consist mostly of empty cell ghosts and broken cell membranes accounted for nearly

all the nystatin originally bound to the cells. On chemical analysis it was found to have a very high content of sterol, low concentration of nucleic acids and a normal content of proteins based on dry weight of these cellular fractions.

The above results seem to indicate that the sterol, which appear to be a component of the cell membrane, is actually the site of binding of nystatin. We might add here that this sterol of *L. donovani*, which has a -OH at the 3rd carbon atom and unsaturation at the 7-8 position, has not yet been fully characterised by us; but it is different from cholesterol or ergosterol. The fact that all the sterol in *L. donovani* was present in the unesterified form (Chatterjee and Ghosh, 1961) indicated that it had a structural rather than a metabolic function—like the unesterified sterol in the membrane of erythrocytes. It may be relevant to recall here that the ability of sterols to antagonise the action of nystatin has been observed by other workers (Lampen *et al.*; 1960), and this has also been confirmed by us. It has further been observed in our laboratory that nystatin has surface-active properties and so a possible explanation of the lytic action of nystatin might be one of simple detergency. But the picture may not be as simple as that for we have found that the action of nystatin was markedly different from that of the cationic detergent CTAB and anionic detergent SDS which also caused a lysis of the cells of *L. donovani*. It is possible that both binding of nystatin on to a receptor site (most probably sterol in nature) on the cell surface and its surface-active properties play a role in the cellular disorganisation of *L. donovani*. Since practically nothing is known regarding the chemical composition and structure of the cell envelopes of hemoflagellates, it would be difficult to hazard any further opinion on the mode of binding of nystatin without adequate experimental data. We are at present trying to characterise the bound form of nystatin which has been isolated from the cells of *L. donovani*, and the details of this work will be published soon.

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REFERENCES

Bradley, S. G. (1958), *Antibiotics and Chemotherapy*, **8**: 282.
Chatterjee, A. N. (1959), Thesis submitted for D. Phil degree of Calcutta University.
Chatterjee, A. N. and Ghosh, B. K., ibid. Abst. No. 27.
Ghosh, B. K., Haldar, D. and Chatterjee, A. N. (1960), *Ann. Biochem. Exptl. Med.*, **20**: 55.

Ghosh, B. K., Haldar, D. and Chatterjee, A. N. (1961), *Ann. Biochem. Exptl. Med.*, **21**: 25.
Ghosh, B. K. and Chatterjee, A. N., Paper presented at the Fourth Indian Microbiological Congress, on 28th March 1961. Abst. No. 26.
Ghosh, B. K. and Chatterjee, A. N. (1961), *Ann. Biochem. Exptl. Med.* (in press)
Lampen, J. O., Arnow, P. M. and Safferman, R. S. (1960), *J. Bact.*, **80**: 200.
Mitchell, P. and Moyle, J. (1956), *Symp. Soc. Gen. Microbiol.*, **6**: 150.
Salton, M. R. J. (1955), *Progr. Biophys.*, **5**: 41.
Spiegelman, S., Aronson, A. I. and Fitz-James, P. C. (1958), *J. Bact.*, **75**: 102.

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